Production and use of a polar lipid-rich fraction containing omega-3 and/or omega-6 highly unsaturated fatty acids from microbes, genetically modified plant seeds and marine organisms
Title: PRODUCTION AND USE OF A POLAR LIPID-RICH FRACTION CONTAINING OMEGA-3 AND/OR OMEGA-6 HIGHLY UNSATURATED FATTY ACIDS FROM MICROBES, GENETICALLY MODIFIED PLANT SEEDS AND MARINE ORGANISMS

Abstract: The production and use, and in particular, the extraction, separation, synthesis and recovery of polar lipid-rich fractions containing eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA(n-3) or DPA(n-6)), arachidonic acid (ARA), and eicosatetraenoic acid (C20:4n-3) from microorganisms, genetically modified seeds and marine organisms (including fish and squid) and their use in human food applications, animal feed, pharmaceutical applications and cosmetic applications.
PRODUCTION AND USE-OF A POLAR LIPID-RICH FRACTION CONTAINING OMEGA-3 AND/OR OMEGA-6 HIGHLY UNSATURATED FATTY ACIDS FROM MICROBES, GENETICALLY MODIFIED PLANT SEEDS AND MARINE ORGANISMS

FIELD OF THE INVENTION

The present invention relates to the fields of production and use, and in particular, the extraction, separation, synthesis and recovery of polar lipid-rich fractions containing eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA(n-3) or DPA(n-6)), arachidonic acid (ARA), and eicosatetraenoic acid (C20:4n-3) from microorganisms, genetically modified seeds and marine organisms (including fish and squid) and their use in human food applications, animal feed, pharmaceutical applications and cosmetic applications.

BACKGROUND OF THE INVENTION

Any discussion of documents, acts, materials, devices, articles and the like is included in the specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

Highly unsaturated fatty acids of the omega-6 and omega-3 series represent a special class of bioactive lipids in that they are important structurally in membranes in the body, but also participate directly and indirectly in communication between cells through the eicosanoid pathways and by their influence of these fatty acids on gene expression. Six of these fatty acids, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA(n-3) or DPA(n-6)), arachidonic acid (ARA), and eicosatetraenoic acid (C20:4n-3) have been shown to be effective in preventing/treating cardiovascular disease, inflammatory disease, immune function imbalances, fertility and some of these fatty acids (ARA, DHA DPA(n-6)) are important structurally in the brain and nervous system. Recent evidence indicates that some highly unsaturated fatty acids may be more bioavailable when supplied in a phospholipid form than in a triglyceride form.

EPA, DHA, and ARA have historically been supplied to the nutritional supplement markets in the form of oil extracted from algae or fish. However recent evidence indicates that some polyunsaturated fatty acids may be more bioavailable in a phospholipid form rather than in a triglyceride form. This may be because of the bipolar nature of phospholipids, making them readily solubilizable in the gut and available for digestion and uptake. This same bipolar property of phospholipids additionally would make these fatty acids more functional in topical applications such as creams and lotions or more soluble in aqueous-based applications such as beverages because of there ability
to participate in emulsification processes. We propose that there may be important advantages in supplying these omega-3 and omega-6 HUFAs in the form of phospholipids and improved processes for recovering polar lipids enriched in these fatty acids are also needed.

Examples of polar lipids include phospholipids (e.g. phosphatidylycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, diphosphatidylglycerols), cephalins, sphingolipids (sphingomyelins and glycosphingolipids), and glycoglycerolipids. Phospholipids are composed of the following major structural units: fatty acids, glycerol, phosphoric acid, and amino alcohols. They are generally considered to be structural lipids, playing important roles in the structure of the membranes of plants, microbes and animals. Because of their chemical structure, polar lipids exhibit a bipolar nature, exhibiting solubility or partial solubility in both polar and non-polar solvents. The term polar lipid within the present description is not limited to natural polar lipids but also includes chemically modified polar lipids. Although the term oil has various meanings, as used herein, it will refer to the triacylglycerol fraction.

One of the important characteristics of polar lipids, and especially phospholipids, is that they commonly contain polyunsaturated fatty acids (PUFAs: fatty acids with 2 or more unsaturated bonds). In many plant, microbial and animal systems, they are especially enriched in the highly unsaturated fatty acids (HUFAs: fatty acids with 4 or more unsaturated bonds) of the omega-3 and omega-6 series. Although these highly unsaturated fatty acids are considered unstable in triacylglycerol form, they exhibit enhanced stability when incorporated in phospholipids.

The primary sources of commercial PUFA-rich phospholipids are soybeans and canola seeds. These biomaterials do not contain any appreciable amounts of highly unsaturated fatty acids unless they have been genetically modified. The phospholipids (commonly called lecithins) are routinely recovered from these oilseeds as a by-product of the vegetable oil extraction process. For example, in the production of soybean or canola oil, the beans (seeds) are first heat-treated and then crushed, ground, and/or flaked, followed by extraction with a non-polar solvent such as hexane. Hexane removes the triacylglycerol-rich fraction from the seeds together with a varying amount of polar lipids (lecithins). The extracted oil is then de-gummed (lecithin removal) either physically or chemically as a part of the normal oil refining process and the precipitated lecithins
recovered. This process however has two disadvantages: (1) the seeds must be heat-treated before extraction with hexane, increasing the processing cost, and increasing undesirable oxidation reactions and denaturing the protein fraction, thereby decreasing its value as a by-product; and (2) the use of the non-polar solvents such as hexane also presents toxicity and flammability problems that must be dealt with.

The crude lecithin extracted in the "de-gumming" process can contain up to about 33% oil (triacylglycerols) along with sterols and glucosides. One preferred method for separating this oil from the crude lecithin is by extraction with acetone. The oil (triacylglycerols) is soluble in acetone and the lecithin is not. The acetone solution is separated from the precipitate (lecithin) by centrifugation and the precipitate dried under first a fluidized bed drier and then a vacuum drying oven to recover the residual acetone as the product is dried. Drying temperatures of 50-70°C are commonly used. The resulting dried lecithins contain approximately 2-4% by weight of oil (triacylglycerols). Process temperatures above 70°C can lead to thermal decomposition of the phospholipids. However, even at temperatures below 70°C the presence of acetone leads to the formation of products that can impair the organoleptic quality of the phospholipids. These by-products can impart musty odors to the product and also a pungent aftertaste.

What is needed is an improved process for effectively recovering polar lipids and phospholipids rich in omega-3 and omega-6 HUFAs from biomaterials that enables the use of these fatty acid in food, nutritional supplement, pharmaceutical and cosmetic applications. Furthermore the fractions are needed as an ingredient in feed for companion animals and in aquaculture.

SUMMARY OF THE INVENTION

In accordance with the present invention, an improved process is provided for recovering polar lipids enriched in omega-3 and/or omega-6 HUFAs from biomaterials such as seeds and microorganisms and the use thereof.

In one aspect, the present invention provides a composition enriched with at least one of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (n-3) (DPA(n-3)), docosapentaenoic acid (n-6) (DPA(n-6)), eicosatetraenoic acid or arachidonic acid (ARA) comprising:

(a) a polar lipid-rich fraction enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA from microbes excluding Chlorophyceae, or from genetically modified seeds; and

(b) a second component selected from the group consisting of an oil, a polar lipid or mixtures thereof.
In one embodiment of the present invention, a method is provided for providing a human, animal or aquaculture organism diet supplement enriched with at least one of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (n-3)(DPAn-3), eicosatetraenoic acid (n-3), docosapentaenoic acid (n-6)(DPAn-6) or arachidonic acid (ARA). The method includes the steps of producing a polar lipid-rich fraction enriched with at least one of EPA, DHA, DPAn-3, DPAn-6, eicosatetraenoic acid and ARA from microbes excluding Chlorophyceae, or from genetically modified seeds; and providing the polar lipid-rich fraction enriched with at least one of EPA, DHA, DPAn-3, DPAn-6, eicosatetraenoic acid and ARA in a form consumable or usable by humans or animals. Preferably, the animal is a companion animal.

In another embodiment of the present invention, a method is provided for treating a deficiency in at least one of EPA, DHA, DPAn-3, DPAn-6, eicosatetraenoic acid or ARA. The method includes the steps of producing a polar lipid-rich fraction enriched with at least one of EPA, DHA, DPAn-3, DPAn-6, eicosatetraenoic acid and ARA from microbes excluding Chlorophyceae, or from genetically modified seeds; and providing the polar lipid-rich fraction enriched with at least one of EPA, DHA, DPAn-3, DPAn-6, eicosatetraenoic acid and ARA to treat the deficiency. The deficiency can result in an inflammatory condition, an immune system imbalance, a cardiovascular disease, a developmental deficit related to nervous system development, a woman's health condition or an infant's health condition.

In another embodiment of the present invention, a method is provided for treating a chronic inflammatory disease state of the lung. The method includes the steps of producing a purified phospholipid fraction enriched with at least one of EPA, DHA, DPAn-3, DPAn-6, eicosatetraenoic acid and ARA from microbes excluding Chlorophyceae, or from genetically modified seeds; blending the phospholipid fraction enriched with at least one of EPA, DHA, DPAn-3, DPAn-6, eicosatetraenoic acid and ARA with at least one of EPA-, GLA- or SDA-rich oils; and producing an aerosol comprising the blend for the treatment of the disease states. The chronic inflammatory disease state of the lung can result in chronic obstructive pulmonary disease (COPD), asthma or cystic fibrosis.

In another embodiment of the present invention, a method is provided for the treatment of skin lesions, induced burn, UV-irradiation or other skin disorders. The method includes the steps of producing a purified phospholipid fraction enriched with at least one of EPA, DHA, DPAn-3, DPAn-6, eicosatetraenoic acid and ARA from microbes excluding Chlorophyceae, or from genetically modified seeds; blending the phospholipid fraction enriched with at least one of EPA, DHA, DPAn-3, DPAn-6, eicosatetraenoic acid and ARA with at least one of EPA-, GLA- or SDA-rich oils; and producing an aerosol comprising the blend for the treatment of the disease states.
and ARA with at least one EPA, GLA- or SDA-rich oil; and producing a lotion or cream for the treatment of the skin disorders.

In another embodiment, the present invention provides for the use of a polar lipid-rich fraction enriched with at least one of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (n-3) (DPA(n-3)), docosapentaenoic acid (n-6) (DPA(n-6)), eicosatetraenoic acid or arachidonic acid (ARA) from microbes or genetically modified seeds for treating a deficiency in at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA.

In another embodiment of the present invention, a method is provided for treating cachexia and severe fat malabsorption. The method includes the steps of producing a purified phospholipid enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA; blending the purified phospholipid enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA with at least one other purified phospholipid; blending the purified phospholipid enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA with at least one DHA, EPA, GLA- or SDA-rich oil; and producing a liquid or dried dietetic product for the treatment of the disease states. The cachexia or severe fat malabsorption can be a result of cancer or Crohn's disease. Preferably, the at least one other purified phospholipid is obtained from the group consisting of soy, rape seed, evening primrose, safflower, sunflower, canola, peanut, egg and mixtures thereof.

In another embodiment of the present invention, a method is provided for the treatment of H. pylori-infection of gastrointestinal tract. The method includes the steps of producing a polar lipid-rich fraction enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA from microbes excluding Chlorophyceae, or from genetically modified seeds; blending the phospholipid fraction enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA with at least one EPA-, GLA- or SDA-rich oil; and producing a fat emulsion or a dietetic product for the treatment of the disease.

In another embodiment of the present invention, a method is provided for providing a composition enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA. The method includes the steps of extracting a polar lipid-rich fraction enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA from microbes excluding Chlorophyceae, or from genetically modified seeds; and mixing the polar lipid-rich fraction enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA with another oil. Preferably, the another oil is
selected from the group consisting of fish oil, microbial oil, vegetable oil, GLA-containing oil, SDA-containing oil or mixtures thereof.

In another embodiment of the present invention, a method is provided for providing a blend of polar lipids enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA. The method includes the steps of extracting a polar lipid-rich fraction enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA from microbes excluding Chlorophyceae, or from genetically modified seeds; and mixing the polar lipid-rich fraction enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA with another polar lipid. Preferably, the another polar lipid is selected from the group consisting of soy polar lipids, rapeseed polar lipids, sunflower polar lipids, safflower polar lipids, canola polar lipids, peanut polar lipids or egg yolk polar lipids and mixtures thereof.

In another embodiment of the present invention, a fat blend is provided enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA. The fat blend includes a polar lipid-rich fraction enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA from microbes or genetically modified seeds; and another oil. Preferably, the another oil is selected from the group consisting of fish oil, microbial oil, vegetable oil, GLA-containing oil, SDA-containing oil and mixtures thereof.

In another embodiment of the present invention, a method is provided for producing a composition of polar lipids enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA. The method includes the steps of extracting an EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA enriched polar lipid-rich fraction from microbes excluding Chlorophyceae, or from genetically modified seeds; and mixing the EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA enriched polar lipid-rich fraction with another polar lipid. Preferably, the another polar lipid is selected from the group consisting of soy polar lipids, rapeseed polar lipids, sunflower polar lipids, safflower polar lipids, canola polar lipids, peanut polar lipids or egg yolk polar lipids and mixtures thereof.

In another embodiment of the present invention, purified phospholipids enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA derived from polar lipid-rich fraction extracted from genetically modified seeds are provided. Preferably, the EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA enriched phospholipid fraction is in a form consumable or usable by human or animals.

In another embodiment of the present invention, a method is provided for providing a human, animal or aquaculture organism diet supplement enriched with at least one of
cis-cis-9-octadecenoic acid (EPA), docosapentaenoic acid (n-3)(DPA(n-3)), eicosatetraenoic acid (n-3) or docosapentaenoic acid (n-6)(DPA(n-6)). The method includes the steps of producing a polar lipid-rich fraction enriched with at least one of EPA, DPA(n-3), DPA(n-6) and eicosatetraenoic acid from microbes excluding Chlorophyceae, or from genetically modified seeds; and providing the polar lipid-rich fraction enriched with at least one of EPA, DPA(n-3), DPA(n-6) and eicosatetraenoic acid in a form consumable or usable by humans or animals.

The polar lipid-rich fraction of the methods or products of can be provided as an ingredient of dietetic, pharmaceutical and cosmetic applications.
The blend of polar lipids of the methods or products of the present invention can be provided as an ingredient of dietetic, pharmaceutical and cosmetic applications.

The purified phospholipids of the methods or products of the present invention can be provided as an ingredient of dietetic, pharmaceutical and cosmetic applications.

Preferably, the marine animals of the methods and products of the present invention are fish, squid, mollusks or shrimp. Preferably, the marine animals are fish or fish eggs from the group including salmon, tuna, haddock, sardines, mackerel, or menhaden.

Preferably, the microbes of the methods and products of the present invention are selected from fungi, microalgae, protozoa or bacteria. More preferably, microbes are;

selected from the Stramenopiles, Thraustochytriales, Chrysophyceae, Xanthophyceae, Bacillariophyceae, Dinophyceae, Phaeophyceae, Rhodophyceae, Euglenophyceae, Cryptophyceae, Oomycetes, Chytridomycetes, or Zygomycetes. More preferably, the microbes are selected from the group of genera consisting of Mortierella, Mucor, Phycomyces, Rhizopus, Pythium, Ochromonas, Nitzschia, Phaeodactylum, Skeletonema, Laminaria, Platymonas, Achyla, Phytophera, Schizochytrium, Thraustochytrium, or Crypthecodinium.

Preferably, the EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid, ARA or mixture thereof employed in the methods and products of the present invention makes up at least two weight percent of the total fatty acids of the polar lipid fraction.

Preferably, the EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid, ARA or mixture thereof employed in the methods and products at the present invention makes up at least five weight percent of the total fatty acids of the polar lipid fraction.

Preferably, the plant seeds or microbes employed in the methods and products to the present invention have been genetically modified to increase their n-3 or n-6 HUFA content.

Preferably, the seeds or microbes used in the methods and products of the present invention have been genetically modified to increase the production of at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA.

Preferably, the seeds employed in the methods and products of the present invention are selected from the group consisting of canola, rapeseed, linseed, flaxseed, sunflower, safflower, peanuts, soybeans or corn. Preferably, the polar lipid-rich fraction is extracted from the seeds and microbes using alcohol.

In an alternative embodiment of the present invention, the polar lipid-rich fraction is derived as a by-product (e.g., by de-gumming) of oil extraction from the seeds using hexane and other non-polar solvents.
Preferably, the polar lipid-rich fraction used in the methods and products of the present invention is extracted from the seeds and microbes by use of gravity or centrifugal extraction technology.

**DETAILED DESCRIPTION OF THE INVENTION**

Because of their bipolar nature, polar lipids (including phospholipids) are of significant commercial interest as wetting and emulsifying agents. These properties may also help make HUFAs in the phospholipids more bioavailable, in addition to enhancing their stability. These properties make phospholipids ideal forms of ingredients for use in nutritional supplements, food, infant formula, pharmaceutical, and cosmetic applications.

Dietary benefits of phospholipids include both improved absorption and improved incorporation. Phospholipids also have a broad range of functionality in the body in that they are important cell membrane constituents, they are good emulsifiers, they can act as intestinal surfactants, serve as a choline source and as a source of HUFAs.

EPA, DHA, and ARA are normally produced for the nutritional supplement market and food uses through the extraction (by cooking) of fish (EPA and DHA) such as menhaden, tuna or salmon, or hexane extraction of fungal biomass (ARA) from the genus Mortierella. The phospholipids in both processes are removed in a later degumming step that produces a waste material comprising a complex mixture of neutral lipids, sterols, glucosides and phospholipids. This material is normally sold to the domestic animal feed industry to dispose of it.

Besides fish and fungal biomass, there are microbial sources of DHA and DPA(n 6). For the present invention, useful microbes can be selected from fungi, microalgae, protozoa or bacteria. These organisms can be selected from the groups including the Stramenopiles, Thraustochytriales, Chrysophyceae, Xanthophyceae, Bacillariophyceae, Dinophyceae, Phaeophyceae, Rhodophyceae, Euglenophyceae, Cryptophyceae, Oomycetes, Chytridomycetes, or Zygomycetes. Useful microbes can also be selected from the group of genera consisting of Mortierella, Mucor, Phycomyces, Rhizopus, Pythium, Ochromonas, Nitzschia, Phaeodactylum, Skeletonema, Platymonas, Achyla, Phytophera, Schizochytrium, Thraustochytrium, or Cryptocodonium. Microorganisms are good sources of phospholipids because they can be grown in culture in a manner that optimizes phospholipid production and minimizes triglyceride (oil) production. On the other hand the methods used in this invention allow both oil and phospholipids to be recovered separately in forms that can be used directly in food, feed, nutritional supplements, cosmetic or pharmaceutical application.

DHA, EPA and ARA phospholipids can be recovered from fish, microalgae, or fungi through the degumming process described above. However as noted this produces a...
complex material containing many other compounds including neutral lipids, sterols, glucosides, etc.

A preferred embodiment of the present invention is to use alcohol and centrifugation to recover the omega-3 and/or omega-6 HUPA-rich phospholipids. Preferred methods for this recovery are described in the following references, which are incorporated by reference herein in their entirety:

i. PCT Application Serial No. PCT/US01/12047, entitled "Method for the Fractionation of Oil and Polar Lipid-Containing Native Raw Materials" filed April 12, 2001;


Once the omega-3 and omega-6 rich phospholipid fractions have been extracted by these preferred processes, they can be used directly as ingredients or they can be purified further and even separated into phospholipid classes by well-known techniques.
such as different forms of chromatography, molecular distillation, and special refining techniques. Polar lipid groups phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, and sphingolipids. The phospholipid rich polar lipids or the purified phospholipid rich fractions can also be mixed with another lipid or oil such as fish lipids, microbial lipids, vegetable lipids, GLA-containing lipids, SDA-containing lipids and mixtures thereof, or be mixed with another phospholipid fraction (lecithin) such as soy or egg yolk lecithin, sunflower lecithin, peanut lecithin or mixtures thereof prior to use as a nutritional supplement, feed or food ingredient. These mixtures of phospholipids can also be incorporated into creams or lotions for topical applications (e.g. treating of skin conditions) or skin lesions induced by burns, UV-irradiation or other skin damaging processes. The mixtures can also be processed to produce a liquid or spray-dried dietetic product or fat emulsion for treating cachexia and severe fat malabsorption or for treatment of *H. pylori* infection of the gastrointestinal tract or incorporated into aerosol sprays for treating chronic inflammatory disease states of the lung (e.g., COPD, asthma, cystic fibrosis).

Advantages of the present invention including providing omega-3 and/or omega-6 HUFAs in a more bioactive and functional form (phospholipid) than the triglyceride form and include a better process (e.g., a) no need for heat treatment; b) no use of toxic solvents (like hexane) and c) no artifacts and off-flavors due to the use of acetone) for recovering these phospholipids from oilseeds and microbes. The EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and/or ARA content of the total fatty acids of the polar lipid fractions and comprise at least two weight percent of the total fatty acids of the polar lipid fraction, more preferably greater than 5 weight percent, more preferably greater than 10 weight percent, and most preferably greater than 20 weight percent of total fatty acids in the polar lipid fraction.

**EXAMPLE**

**Example 1**

Phospholipids were extracted from salmon, salmon roe, Black tiger prawns, squid, *Schizochytrium* sp, and the total fatty acid content of the phospholipids was determined by gas chromatography. The results are presented in Table 1. As can be observed the phospholipid fraction of these biomaterials can be used to deliver omega-3 and/or omega-
6 HUFAs and in this form these bioactive fatty acids should be more stable, more bioavailable, and more functional.

Table 1. Total fatty acid content of phospholipids extracted from 4 types marine animal products and forma microbial source.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Salmon Roe</th>
<th>Salmon Roe</th>
<th>Tiger Prawn</th>
<th>Squid</th>
<th>Schizochytrium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PL's % TFA</td>
<td>PL's % TFA</td>
<td>PL's % TFA</td>
<td>PL's % TFA</td>
<td>PL's % TFA</td>
</tr>
<tr>
<td>MYRISTATE C14:0</td>
<td>2.99</td>
<td>4.58</td>
<td>1.14</td>
<td>1.87</td>
<td>2.66</td>
</tr>
<tr>
<td>MYRISTOLEATE C14:1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.05</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>PALMITATE C16:0</td>
<td>26.48</td>
<td>29.661</td>
<td>25.44</td>
<td>30.37</td>
<td>28.25</td>
</tr>
<tr>
<td>PALMITOLEATE C16:1</td>
<td>2.86</td>
<td>4.38</td>
<td>1.55</td>
<td>0.23</td>
<td>0.62</td>
</tr>
<tr>
<td>STEARATE C18:0</td>
<td>5.74</td>
<td>6.19</td>
<td>13.59</td>
<td>4.41</td>
<td>1.75</td>
</tr>
<tr>
<td>OLEATE C18:1</td>
<td>9.74</td>
<td>13.36</td>
<td>13.33</td>
<td>1.86</td>
<td>6.99</td>
</tr>
<tr>
<td>LINOLEATE C18:2n6</td>
<td>3.14</td>
<td>1.05</td>
<td>12.51</td>
<td>0.32</td>
<td>2.46</td>
</tr>
<tr>
<td>GAMMA LINOLENATE C18:3n6</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1.09</td>
</tr>
<tr>
<td>ARACHIDATE C20:0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.74</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>LINOLENATE C18:3n3</td>
<td>0.49</td>
<td>0.22</td>
<td>0.54</td>
<td>0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>OCTADECATETRAENOATE C18:4</td>
<td>0.50</td>
<td>0.33</td>
<td>0.08</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>EICOSANOATE-11</td>
<td>0.75</td>
<td>1.07</td>
<td>0.85</td>
<td>5.54</td>
<td>0.00</td>
</tr>
<tr>
<td>EICOSADIENOATE-11,14</td>
<td>0.00</td>
<td>0.00</td>
<td>0.56</td>
<td>0.34</td>
<td>0.00</td>
</tr>
<tr>
<td>BEHENATE C22:0</td>
<td>0.00</td>
<td>0.00</td>
<td>1.35</td>
<td>0.08</td>
<td>0.00</td>
</tr>
<tr>
<td>EICOSATRIENOATE C20:3n3</td>
<td>1.01</td>
<td>3.58</td>
<td>0.07</td>
<td>0.42</td>
<td>1.61</td>
</tr>
<tr>
<td>ARACHIDONATE C20:4 n6</td>
<td>0.89</td>
<td>0.56</td>
<td>5.54</td>
<td>1.70</td>
<td>1.18</td>
</tr>
<tr>
<td>ERUCATE C22:1</td>
<td>0.21</td>
<td>0.00</td>
<td>0.04</td>
<td>1.41</td>
<td>0.00</td>
</tr>
<tr>
<td>EICOSAPENTAENOATE C20:5n3</td>
<td>9.50</td>
<td>9.39</td>
<td>8.47</td>
<td>14.71</td>
<td>5.11</td>
</tr>
<tr>
<td>LIGNOCERATE C24:0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.56</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>NERVONATE C24:1</td>
<td>1.61</td>
<td>1.65</td>
<td>0.73</td>
<td>0.82</td>
<td>0.00</td>
</tr>
<tr>
<td>DOCOSAPENTAENOATE n-6 C22:5n6</td>
<td>0.00</td>
<td>0.00</td>
<td>0.45</td>
<td>0.22</td>
<td>17.68</td>
</tr>
<tr>
<td>DOCOSAPENTAENOATE n-3 C22:5n3</td>
<td>3.16</td>
<td>4.40</td>
<td>0.55</td>
<td>0.44</td>
<td>0.00</td>
</tr>
<tr>
<td>DOCOSAHEXAENOATE C22:6n3</td>
<td>30.92</td>
<td>19.50</td>
<td>11.86</td>
<td>35.54</td>
<td>30.61</td>
</tr>
</tbody>
</table>

The present invention, in various embodiments, includes components, methods, processes, systems and/or apparatus substantially as depicted and described herein, including various embodiments, subcombinations, and subsets thereof. Those of skill in the art will understand how to make and use the present invention after understanding the present disclosure. The present invention, in various embodiments, includes providing devices and processes in the absence of items not depicted and/or described herein or in various embodiments hereof, including in the absence of such items as may have been
used in previous devices or processes, *e.g.*, for improving performance, achieving ease and/or reducing cost of implementation.

The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, *e.g.*, as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those Claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

It is to be noted that, throughout the description and claims of this specification, the word 'comprise' and variations of the word, such as 'comprising' and 'comprises', is not intended to exclude other additives, components, integers or steps.
The claims defining the invention are as follows:

1. A composition enriched with at least one of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (n-3) (DPA(n-3)), docosapentaenoic acid (n-6) (DPA(n-6)), eicosatetraenoic acid or arachidonic acid (ARA) comprising:
   (a) a polar lipid-rich fraction enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA from microbes excluding Chlorophyceae, or from genetically modified seeds; and
   (b) a second component selected from the group consisting of an oil, a polar lipid or mixtures thereof.

2. The composition of claim 1, wherein said second component is selected from the group consisting of fish oil, microbial oil, vegetable oil, EPA-containing oil, GLA-containing oil, SDA-containing oil and mixtures thereof.

3. The composition of claim 1, wherein said second component is selected from the group consisting of soy polar lipids, rape seed polar lipids, sunflower polar lipids, safflower polar lipids canola polar lipids, peanut polar lipids or egg yolk polar lipids and mixtures thereof.

4. The composition of claim 1, wherein the at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA are derived from polar lipid-rich fraction extracted from genetically modified seeds and the second component is purified phospholipids.

5. The composition of claim 4, wherein said composition is in a form consumable or usable by humans or animals.

6. A method for providing the composition of Claim 1, comprising the steps:
   (a) extracting a polar lipid-rich fraction enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA from microbes excluding Chlorophyceae, or from genetically modified seeds; and
   (b) mixing said polar lipid-rich fraction enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA with a second component selected from the group consisting of an oil, a polar lipid or mixtures thereof.
7. The method of claim 6, wherein said second component is selected from the group consisting of fish oil, microbial oil, vegetable oil, EPA-containing oil, GLA-containing oil, SDA-containing oil or mixtures thereof.

8. The method of claim 6, wherein said second component is selected from the group consisting of soy polar lipids, rape seed polar lipids, sunflower polar lipids, safflower polar lipids, canola polar lipids, peanut polar lipids or egg yolk polar lipids and mixtures thereof.

9. A method for providing a human, animal or aquaculture organism diet supplement enriched with at least one of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (n-3)(DPA(n-3)), eicosatetraenoic acid (n-3), docosapentaenoic acid (n-6)(DPA(n-6)) or arachidonic acid (ARA) comprising the steps:
   (a) producing purified phospholipids of claim 4; and
   (b) providing said purified phospholipids enriched with at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid and ARA in a form consumable or usable by humans or animals.

10. The method of claim 9, wherein the animal is a companion animal.

11. The composition according to any one of claims 1 to 5 or a product of the method of any one of claims 6 to 10, as an ingredient of dietetic, pharmaceutical or cosmetic applications.

12. The method, composition or product of any preceding claim, wherein said microbes are selected from fungi, microalgae excluding Chlorophyceae, protozoa or bacteria.

13. The method, composition or product of any preceding claim, wherein said microbes are microalgae selected from the Stramenopiles, Thraustochytriales, Chrysophyceae, Xanthophyceae, Bacillariophyceae, Dinophyceae, Phaeophyceae, Rhodophyceae, Euglenophyceae, Cryptophyceae, Oomycetes, Chytridomycetes, or Zygomycetes.
14. The method, composition or product of any preceding claim, wherein said microbes are selected from the group of genera consisting of Mortierella, Mucor, Phycomyces, Rhizopus, Pythium, Ochromonas, Nitzschia, Phaeodactylum, Skeletonema, Laminaria, Platymonas, Achyla, Phytophthora, Schizochytrium, Thraustochytrium, or Cryptothecodinium.

15. The method, composition or product of any preceding claim, wherein EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid, ARA or mixture thereof comprises at least two weight percent of the total fatty acids of the polar lipid fraction.

16. The method, composition or product of any preceding claim, wherein EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid, ARA or mixture thereof comprises at least five weight percent of the total fatty acids of the polar lipid fraction.

17. The method, composition or product of any preceding claim, wherein said plant seeds or microbes have been genetically modified to increase their n-3 or n-6 HUFA content.

18. The method, composition or product of any preceding claim, wherein said seeds or microbes have been genetically modified to increase the production of at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA.

19. The method, composition or product of any preceding claim, wherein said seeds are selected from the group consisting of canola, rape seed, linseed, flaxseed, sunflower, safflower, peanuts, soybeans or corn.

20. The method, composition or product of any preceding claim, wherein said polar lipid-rich fraction is extracted from said seeds and microbes using alcohol.

21. The method, composition or product of any preceding claim, wherein said polar lipid-rich fraction is derived as a by-product of oil extraction from said seeds using hexane or other non-polar solvent.

22. The method, composition or product of any preceding claim, wherein said polar lipid-rich fraction is extracted from said seeds and microbes by use of gravity or centrifugal extraction technology.
23. Use of a polar lipid-rich fraction enriched with at least one of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (n-3) (DPA(n-3)), docosapentaenoic acid (n-6) (DPA(n-6)), eicosatetraenoic acid or arachidonic acid (ARA) from microbes excluding Chlorophyceae, or from genetically modified seeds for treating a deficiency in at least one of EPA, DHA, DPA(n-3), DPA(n-6), eicosatetraenoic acid or ARA.

24. The use according to claim 23, wherein said deficiency results in an inflammatory condition, an immune system imbalance, a cardiovascular disease, a developmental deficit related to nervous system development, a woman's health condition or an infant's health condition.

25. The use of the polar lipid-rich fraction according to claim 23 in the preparation of a medicament for treating the deficiency according to claim 23 or claim 24.

26. Use of the composition according to claim 1, wherein the second component is selected from the group consisting of at least one of EPA-, GLA- or SDA-rich oils, for treating a chronic inflammatory disease state of the lung.

27. The use according to claim 26, wherein the chronic inflammatory disease state of the lung is COPD, asthma or cystic fibrosis.

28. The use of the composition according to claim 26 in the preparation of a medicament for treating the disease according to claim 26 or claim 27.

29. The use according to claim 28, wherein the medicament is an aerosol.

30. Use of the composition according claim 1, wherein the second component is selected from the group consisting of at least one EPA-, GLA- or SDA-rich oil for the treatment of skin lesions, induced burn, UV-irradiation or other skin disorders.

31. The use of the composition according to claim 30 in the preparation of a medicament for treating the disease according to claim 30.

32. The use according to claim 31, wherein the medicament is a lotion or cream.
33. Use of the composition according to claim 1, wherein the second component comprises a polar lipid and at least one DHA, EPA, GLA- or SDA-rich oil for treating cachexia or fat malabsorption.

34. The use according to claim 33, wherein the cachexia or fat malabsorption is a result of cancer or Crohn's disease.

35. The use according to claim 34, wherein the polar lipid in the second component is obtained from the group consisting of soy, rape seed, evening primrose, safflower, sunflower, canola, peanut, egg and mixtures thereof.

36. The use of the composition according to claim 33 or claim 35 in the preparation of a medicament for treating the disease according to any one of claims 33 to 35.

37. The use according to claim 36, wherein the medicament is a liquid or dried dietetic product.

38. Use of the composition according to claim 1, wherein the second component comprises at least one EPA-, GLA- or SDA-rich oil for the treatment of *H. pylori*-infection of gastrointestinal tract.

39. The use of the composition according to claim 38 in the preparation of a medicament for treating the disease according to claim 38.

40. The use according to claim 39, wherein the medicament is a fat emulsion or a dietetic product.